REMARKS

Claims 1, 10, 11, 15, 16, 18-21 and 25-29 are pending in the application. All of the claims have been finally rejected under Section 103 on new grounds: Claims 1. 10, 11, 15, 16, 19-21, 25, 26, 28 and 29 are now rejected under Section 103 based on Rutanen (U.S. 2005/0136490) in view of Boehringer (WO 98/39657). Claim 18 is now rejected over Rutanen in view of Boehringer and in further view of Cole (U.S. 6,656,745), while claim 27 is now rejected over Rutanen in view of Boehringer and in further view of Dart ("Rate Change of Serial β-Human Chronionic Gonadotropin Values ..." from *Annals of Emerging Medicine*, Vol. 34, No. 6 (1999) 703-710.

In addition, claims 10, 15, 19, 20 and 25 are rejected under Section 112 as indefinite. The above amendment to claims 10, 20 and 25 places all of the claims so rejected in compliance with Section 112. In addition, claim 1 is amended in order to correct an error introduced in the prior amendment. It was intended that the text *"to induce, at the first time"* was to be deleted, and this text is now deleted without introduction of any new issues. As best understood from the final office action, the examination of claim 1 did not give any weight to that language which should have been deleted. Thus the actual deletion of the subject language brings the claim more fully into compliance with Section 112 without introducing any new issues.

It is respectfully submitted that none of the above amendments introduce any new issues which require further consideration or searching. Because these corrections place the application in better condition for allowance or appeal, this amendment should be entered.

Applicant and applicant's attorney thank the Examiner for the assistance provided in the telephone discussion of September 15, 2009, at which time the Examiner explained more specifically how the Rutanen reference has been relied upon to reject all of the independent claims as part of the combination of references applied under Section 103. During the telephone discussion it was explained to the applicant's attorney that the rejection of the claims is based in part on Table 2 and par 41 of the Rutanen reference. At the time of the telephone discussion it did not appear conclusive that the tests by which the data in Table 2 of the reference were obtained involved portable test units of the type which provide visually observable responses based on capillary flow of a sample . The argument which follows demonstrates that reliance

upon the Rutanen reference in combination with the Boehringer reference provides no more basis for obviousness than any of the prior rejections which have relied upon the Boehringer reference.

As discussed in the patent specification, it is known in the prior art to track temporally variable concentrations of chemical species in fluids. However, assay methodologies for doing such have been relatively complex, normally requiring analytical or quantitative procedures which procedures require time-intensive laboratory analysis of blood or other fluids. See pages 1 and 2 of the application which cite use of a Solid Phase **E**nzyme **L**inked **I**mmuno**s**orbent **A**ssay (ELISA) at designated time intervals to monitor changes in hCG concentration. The ELISA tests require adding serum to an hCG microtiter well, and when hCG is present, it binds to an antibody in the well. When another antibody, i.e., a label, is added, it binds to the antigen-antibody complex, allowing for coloration that facilitates visual observation and spectrophotometric analysis. Concentration of hCG can then be determined based on color intensity. By repeating the ELISA test at time intervals, shifts in levels of hCG can be determined. Such quantitative measurement can reveal a decrease in hCG level, which may be a warning sign of a pregnancy complication.

In view of this prior art, the invention according to claim 10 provides for use of multiple test units each containing a receiving zone and two or more regions each responsive to capillary flow of analyte at a different and measurably distinguishable sensitivity level. Each distinguishable sensitivy level is indicative of presence of a different amount of analyte in the source. On different occasions in time different samples from the same source may be brought into contact with different ones of the test devices to determine whether a change in health condition occurs.

A rejection of claim 10 must identify a disclosure wherein tests are performed on at least two samples obtained from a source at two different times, i.e., comparable to the above example of an ELISA test, **but**, according to claim 10, both of the tests are performed on lateral flow test units wherein each of two or more regions are

"responsive to analyte migrating from the receiving zone by capillary flow ... [with] the two or more regions on each test unit defining multiple measurably distinguishable sensitivity levels ..."

The only disclosure in the prior art combination of Rutanen and Boehringer, that concerns tests performed on samples obtained from a source at two different times, is in relation to Table 2 of the Rutanen reference. That table provides concentrations of secretions from patients. Data was obtained for each patient at multiple different times during a gestation period. In the telephone discussion with the Examiner it was understood that this Table 2 and paragraph 41 of the Rutanen reference were construed as disclosure of multiple test units each comprising a nitrocellulose membrane test strip having two IGFBP-1 antibodies in accord with Example 3 of Rutinen (see paragraph 80); and that such membrane test strips were used for acquiring the data of Table 2.

Applicant respectfully disagrees with this assessment. It is apparent from the written description of the Rutanen reference that nitrocellulose membrane test strips in accord with Example 3 of Rutanen were not used to acquire the data of Table 2. Specifically, the reference states at paragraph 41 that

"The IGFBP-1 concentrations shown in said tables were obtained using secretion samples extracted in 0.5 ml of buffer and the quantitative test IGFBP-1 IEMA Test Cat 10831 (Oy Medix Biochemica Ab, Finland)."

The attached excerpt, pages 105 – 106, from Anderson and Cockayne, Clinical Chemistry: Concepts and Applications, Wavelend Press 2007, confirms that an IEMA test is not a lateral flow test. For example, as explained at page 105 of this reference, IEMA tests are a form of ELISA test wherein

1. polystyrene beads are coated with antigen specific to the target antibody by absorption
2. sample solutions containing the antigen are incubated with the coated beads
3. the amount of bound antigen is quantitated with an enzyme-conjugated antibody
4. a wash step is performed to remove unbound antigen
5. color change is proportional to the amount of bound enzyme-conjugate antigen

As stated at page 106, the bound enzyme-conjugate antigen on the substrate enables measurement based on color, and the assay may be performed in a laboratory with a spectrophotometer.

In summary, IEMA tests are characterized by separation steps, quantitation based on color density and use of laboratory equipment. They are not lateral flow tests. In accord with the above description of an IEMA test, paragraph 53 of the Rutinen reference describes an IGFBP-1 test involving a plastic bead wherein enzyme-labelled antibody and the antibody-coated bead are placed in a test tube and incubated, during which time a color may develop. After washing a quantitative value of concentration may be determined photometrically.

While the Rutanin reference does describe use of a membrane strip at paragraph 80, there is no disclosure of using two such strips, one for a first sample provided at a first time and the other for a second sample provided at a second time

wherein an abnormal difference between visually observable responses induced in the first test unit at the first time and induced in the second test unit at the second time, each based on binding of an antigen and an antibody, provides information about whether an adverse change in the health condition has occurred between the two times.

Rather, the Rutanen reference only suggests a prior art laboratory-based test analogous to the ELISA test for hCG noted in applicant's specification.

Further, the data of Table 2 in the Rutanen reference confirms use of a test procedure that indicates actual concentrations rather a test procedure that indicates whether threshold values of concentration have been exceeded. For this reason, the combination kit described in paragraph 66 of the reference would not be suitable for acquiring the data of Table 2. In fact, by all appearances the test used for acquiring the data of Table 2 measures at least eight different levels ranging from 0.6 up to 8.8, and these appear to be arbitrary levels. That is, the data in Table 2 corresponds to actual measured concentrations rather than predetermined threshold levels.

Applicant submits that the above-noted distinctions, while presented with regard to claim 10, provide a basis for allowance of all of the claims. That is, each of the other independent claims is allowable over the combination of Rutanen in view of Boehringer for reasons similar to those noted for claim 10. None of the prior art provides any recognition that one might use test

units or devices, which operate on the basis of lateral flow or capillary flow, for comparing visually observable responses from different samples acquired at different times.

For all of the above reasons it is submitted that the final rejection is in error and allowance of the application is requested.

Respectfully submitted,

Dated: November 5, 2009 ____/Ferdinand M. Romano/_____

Ferdinand M. Romano
Registration No. 32,752
(407) 926-7722

Beusse Wolter Sanks Mora & Maire, P.A.
390 N. Orange Avenue, Suite 2500
Orlando, FL 32801

One Enclosure: two pages of text
from Clinical Chemistry: Concepts and Applications

Clinical chemistry: concepts and applications By Shauna C. Anderson, Susan Cockayne

five binding sites can then function as acceptors for the biotin-labeled enzyme. This procedure can be shortened by using biotin-labeled antibody and enzyme-labeled avidin.

Buffers are used to maintain reaction pH and ion concentration, for sample dilutions or as diluents in reconstituting the lyophilized assay reagents.

Enzymes and substrate combinations include the enzyme horseradish peroxidase, its substrate hydrogen peroxide, and chromogen *o*-phenylenediamine. The product of this reaction produces a strong yellow-orange color. Other enzymes include β-galactosidase and its substrate *o*-nitrophenyl-β-D-galactopyranoside, which is converted to a yellow nitrophenolate ion, and alkaline phosphatase and its substrate *p*-nitrophenyl phosphate, which is converted to nitrophenolate ion.

Stopping reagent 1-N sulfuric acid is used to inhibit enzyme activity and stabilize the final colored reaction product.

Competitive-Binding Assays

A solid-phase competitive-binding reaction ELISA is frequently used for the detection of antigen or hapten. In this type of assay, unlabeled ligand (patient sample) competes with enzyme-conjugated ligand for a limited number of immobilized antibody-binding sites. After a brief incubation, separation of bound and free enzyme-conjugated ligand is accomplished using the solid-phase separation technique described under RIA in this chapter. Substrate is added and enzyme present in the bound fraction converts substrate to a colored product. The amount of product formed is inversely related to the concentration of the unlabeled ligand in the test sample. The absorbances of the standards, controls, and test samples are determined using a spectrophotometer set at the appropriate wavelength within 2 hours after the addition of the stopping reagent. The reference wells containing only enzyme-conjugated stopped by the addition of the stopping reagent. Enzyme activity is decreased in the presence of antibody in the test sample so that within the detectable range of the assay, the greater the concentration of the antibody, the less the absorbance of the sample.

Immunoenzymetric Assays

NONCOMPETITIVE ASSAYS

Immunoenzymetric assays (IEMA), also referred to as the sandwich technique, are the most commonly used ELISA methods for the detection of antigens bearing at least two antigenic determinants. An excess of antibody, specific for the antigen, frequently monoclonal, is adsorbed onto polystyrene beads. These antibodies are specific for a unique antigenic determinant on the antigen of interest. Sample solutions are incubated with the antibody-coated beads so that all of the antigen present in the test sample will be bound by the immobilized antibody. After removal of unbound material, the amount of antigen bound to the antibody is quantitated by adding an enzyme-conjugated antibody that recognizes a second antigenic determinant on the same antigen. Following a second incubation and separation step, the enzyme activity remaining associated with the sandwich is determined by the addition of the substrate. Product formation is directly related to the amount of antigen in the standards, controls, and test samples. If the second antibody is monoclonal, the antibody reagents can be added simultaneously and only one wash step is required.

The color change is proportional to the amount of antigen in the test solution. A standard curve is obtained by plotting the average absorbance value of each set of duplicate standards versus the antigen concentration contained in each

Clinical chemistry: concepts and applications By Shauna C. Anderson, Susan Cockayne

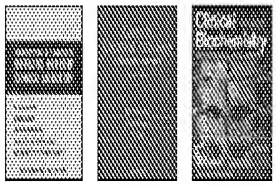

FIGURE 7-7

ELISA. **a.** A limited number of antibody (Ab) molecules attached to polystyrene beads are reacted with antigen (Ag) and enzyme-conjugated antigen. These antigens compete for binding and complexes are formed. **b.** The beads are washed to remove unbound antigen, enzyme substrate is added, and the bound enzyme-conjugate antigen converts the substrate to measurable colored product.

standard on linear graph paper. This produces a straight line. Concentrations of antigen in control and test samples duplicates run concurrently with the standards can be determined from the curve using the average absorbance values. Alkaline phosphatase is the enzyme of choice since it catalyzes a single hydrolysis step and a linear enzymatic response curve is obtained.

Advantages and Disadvantages

The advantages of using enzyme-conjugated antigens and antibodies are that they can be stored under sterile conditions and used for years without any appreciable loss of their enzymatic and immunologic activities. The use of enzymes as labels entails minimal risk of contamination or pollution and the procedures are relatively easy to perform. There is no need for costly equipment and the assay can be performed in laboratories equipped with simple spectrophotometers. The specificity of the assay depends, as does that of RIA, on the specificity of the antibodies used. Similar to the IRMA, the IEMA uses an excess of labeled antibody. Because the label is an enzyme and not a radioisotope, the IEMA is not limited by how much labeled antibody can be added to each assay tube. Therefore, the IEMA can be used to detect antigen over a wider range of concentration. The competitive and noncompetitive methods are highly sensitive, detecting picogram quantities of hormones and other substances. Disadvantages are that most of the assays require multiple incubation and separation steps and take longer to perform than either RIA or IRMA, increasing the chance of error. Automation has helped to alleviate these problems, reducing carryover errors, increasing precision through robotics and reducing turnaround times.

Laboratory Applications

ELISAs are available for the measurement of factor VIII–related antigen, tumor markers such as carcinoembryonic antigen, alpha-fetoprotein, human chorionic gonadotropin, and antibodies (IgG and IgM) produced by the host in response to various viral infections. During the last decade, ELISA wild-phase assays have become the standard method of screening for hepatitis (hepatitis A, B, C, D, and E (HAV, HBV, HCV, HDV, HEV, respectively), antibodies and B surface or core antigens) and the human immunodeficiency virus (HIV). ELISAs for HIV antibodies (Electronucleonics, Inc., or Biorack Laboratories, Rockville, MD) are confirmed by Western blot. In addition to HIV antigens, other viral antigens, cytomegalovirus